

Personalised Therapeutic Strategies in Paediatric Haematopoietic Stem Cell Transplantation

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CERTIFICATE OF ORIGINAL AUTHORSHIP

I, **Felicity Anna Wright** declare that this thesis, is submitted in fulfilment of the requirements for the award of **Doctor of Philosophy (Pharmacy)**, in the **Graduate School of Health** at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis. This document has not been submitted for qualifications at any other academic institution. This research is supported by the Australian Government Research Training Program.

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Pharmacogenetic analysis of thiotepa based conditioning regimens in paediatric acute lymphoblastic leukaemia

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Preface

Cancer is the leading cause of childhood death by disease.

Acute Lymphoblastic Leukaemia (ALL) is the most common paediatric malignancy. In high-risk paediatric ALL, chemotherapy alone may not provide a cure, in these patients haematopoietic stem cell transplantation is offered with curative intent.

This study aims to use transplant related outcomes and pharmacogenomics to lay the foundations for personalised dosing for thiotepa in paediatric haematopoietic stem cell transplantation to reduce toxicity and improve overall survival.

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Version: 4 | Date: 10 August 2018

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Abbreviations

A: adenine

aGVHD: Acute Graft Versus Host Disease

ALL: Acute lymphoblastic leukaemia

BMT: blood and marrow transplant; bone marrow transplant

C: Cytosine

CB or UCB: cord blood, umbilical cord blood

cGVHD: Chronic GVHD

CMV: Cytomegalovirus

CNS: Central Nervous System

CR: Complete Remission

CR1: Complete Remission 1

CR2: Complete Remission 2

CR3: Complete Remission 3

CY: Cyclophosphamide

CYP: Cytochrome P450

CYP P450: Cytochrome P450

CYP2B6: Cytochrome P450 Family 2 Subfamily B Isoform 6

CYP3A4: Cytochrome P450 Family 3 Subfamily A Isoform 4

CYP3A5: Cytochrome P450 Family 3 Subfamily A Isoform 5

DCBT: Double Cord Blood Transplant

DNA: Deoxyribonucleic Acid

G: Guanine

GCSF: Granulocyte colony stimulating factor

GST: Glutathione-S-Transferase

GST A: Glutathione-S-Transferase Isoenzyme Alpha

GST A1: Glutathione-S-Transferase Isoenzyme Alpha Subfamily 1

GST M: Glutathione-S-Transferase Isoenzyme Mu

GST M1: Glutathione-S-Transferase Isoenzyme Mu Subfamily 1

GST P: Glutathione-S-Transferase Isoenzyme Pi

GST P1: Glutathione-S-Transferase Isoenzyme Pi Subfamily 1

GST T: Glutathione-S-Transferase Isoenzyme Theta

GST T1: Glutathione-S-Transferase Isoenzyme Theta Subfamily 1

GST T2: Glutathione-S-Transferase Isoenzyme Theta Subfamily 2

GST Z: Glutathione-S-Transferase Isoenzyme Zeta

GST Z1: Glutathione-S-Transferase Isoenzyme Zeta Subfamily 1

GVHD: Graft Versus Host Disease

HC: Haemorrhagic Cystitis

HCT: hematopoietic cell transplant

HLA: Human Leukocyte Antigen

HSV: Herpes Simplex Virus

HWE: Hardy Weinberg Equilibrium

IgG: Immunoglobulin G

IP: Interstitial Pneumonitis

MPAL: Mixed Phenotype Acute Leukaemia

MUD: Matched Unrelated Donor, now commonly called URD

MUD-MARROW: Matched Unrelated Donor Marrow

MUD-PBSC: Matched Unrelated Donor Peripheral Blood Stem Cells

NCBI: National Center for Biotechnology Information

NPGT500: Neutrophil engraftment

PBSCT: peripheral blood stem cell transplant

PLAT20: Platelet engraftment

rs#/rsID: Reference SNP cluster ID

SCT: stem cell transplant

SNP: Single Nucleotide Polymorphism

SNPID: Single Nucleotide Polymorphism ID

T: thymine

TBI: Total Body Irradiation

TEPA: Triethylenephosphoramidate, N,N',N''-triethylenephosphoramidate

TT: Thiotepa, N,N',N''-triethylenethiophosphoramidate

URD: Unrelated Donor

VOD: Veno-Occlusive Disease

Abstract

Background: Haematopoietic stem cell transplant (HCT) is offered with curative intent to high-risk patients with acute lymphoblastic leukaemia (ALL). The polyfunctional alkylating agent thiotepa is a preparative regimen agent that provides profound myelosuppression and facilitates engraftment. There is a wide range of variability in CYP2B6, CYP3A4 and glutathione-S-transferase (GST) mediated thiotepa metabolism as well an inducible process in the presence of other drugs. This leads to variability in alkylation and potential effectiveness as a preparative regimen agent.

Aim: Identify genetic variants in key metabolising enzymes that impact drug efficacy and toxicity of thiotepa as conditioning chemotherapy in paediatric ALL transplant patients.

Methods: A retrospective pharmacogenomic experiment using stored marrow samples evaluated the association between HCT outcomes and single nucleotide polymorphisms (SNPs) of drug metabolising enzymes. Time to event analyses determined the association between SNPs and transplant outcomes. Multivariate analysis was performed taking significant p values from univariate analysis and applying a regression model using stepwise elimination.

Results: 55 patients received a thiotepa based regimen for first HCT for ALL between 2000-2017 and had an available tumour bank sample. Eight unique SNPs for nine outcomes were identified to have an association with survival, engraftment and toxicity.

Overall Survival was 78.18% at median 50 months follow up and was reduced in rs2740574 (T;T) 76.9%, compared to 100% in CYP3A4*1B carrier rs2740574 (C;T) (p=0.001). rs776746 encoding non-functional CYP3A5*3 had a detrimental effect on leukaemia free survival (LFS) with 67.7% LFS in

rs776746 (C;C) CYP3A5*3/*3 compared to 87.5% LFS for (C;T) CYP3A5*1/*3 and 100% LFS for rs776746 (T;T) CYP3A5*1/*1 compared to 70.9% overall ($p = 0.021$). LFS was also reduced in rs1046428 a missense variant SNP associated with glutathione-S-Transferase Z1 ($p=0.049$).

The median time to neutrophil engraftment was 19 days. GSTT2 rs140188 (C;G) was associated with accelerated engraftment ($p = 0.026$). The median time to platelet engraftment was 29 days.

rs8192709 (C;T) CYP2B6*1/CYP2B6*2 demonstrated delayed platelet engraftment, median 47 days ($p = 0.000385$). Mismatch, donor gender and rs8192709 (C;T) were independent risk factors for delayed platelet engraftment ($p = 0.00003$).

Due to extensive and rapid distribution into the CNS, a hallmark toxicity of thiotepea is CNS adverse events. rs3211371 encoding CYP2B6*5 resulted in reduced CNS toxicity compared to CYP2B6*1 ($p=0.01$). There was a concerning trend to increased CNS relapse in the *5 allele however this wasn't significant ($p = 0.0546$).

Conclusion: This study identified thiotepea as a potential target for pharmacogenomic guided dosing in the paediatric HCT setting. Thiotepea efficacy and toxicity are modified by key polymorphisms in drug metabolising enzymes. The identification of SNPs in drug metabolising enzymes offers an opportunity to tailor drug therapy based on the pharmacogenome of the transplant recipient and lays the foundation for personalised therapeutic dosing strategies in high risk leukaemia.